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Nanoparticle labels for pathogen detection through nucleic acid amplification tests

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Abstract

Magnetic nanoparticles and surface enhanced Raman scattering (SERS) active nanoparticles were coated with short chain DNA tags. These were then used to identify a target bacterial DNA sequence. The tags function as primers in a standard PCR reaction with the reverse primers and forward primers on the SERS-nanoparticles and magnetic-nanoparticles respectively. During the PCR cycles a composite nanostructure is formed that is both magnetically responsive and SERS active. After magnetic trapping the intensity of the SERS signal can be related back to the concentration of the target DNA. A test assay was performed that showed a detection limit (based on the signal to noise ratio) of less than 3 zeptomole (41 pg/L). For comparison a PCR assay based on the standard SYBR Green method was performed. This used the same primers and target DNA and had a detection limit of 10 attomoles (138 ng/L), 3000 times less sensitive. The work documents the proof of principle study and shows for the first time the use of SERS-NP labels in the quantification of nucleic acid amplification tests (NAATs) and PCR reactions.

Key Words: Nanoparticle, Raman, Magnetic, DNA, Primer, PCR, Nucleic acid

Manuscript Text

1. Background

Polymerase chain reaction (PCR) is the gold standard for nucleic acid (NA) based identification of pathogens, however, more recently, Real-Time PCR has started to challenge this position.[1] Compared to standard PCR the technique is faster, more sensitive and offers quantitative as well as qualitative results. In only a few years the technique has gone from the development stage to full market acceptance and is now the method of choice particularly in the area of infectious disease diagnosis, cancer diagnosis and gene transcription.[2] This rapid uptake highlights the need for improved assay performance and the willingness of the scientific community to accept such improvements. Over the last few years several studies have combined nanoparticles with PCR in attempts to enhance various aspects of the technique.[3,4] The simplest approach is to use the nanoparticles as an additive to improve the PCR efficiency. Several groups have reported this kind of application and have shown that gold nanoparticles (AuNPs) can both improve the PCR efficiency and also reduce the efficiency depending on the experimental conditions. Li *et. al.* suggested that the use of AuNPs improves the thermal properties of the PCR solution by affecting the heat transfer.[5] They used standard AuNPs without oligonucleotides attached. Huang *et. al.* showed that the nanoparticle surface area was the most important feature for this phenomenon.[6] It has also been shown that under certain conditions AuNPs can also reduce the PCR efficiency by inhibiting the Taq enzyme on the nanoparticle surface.[7-9] Other nanoparticles have also been used in applications associated with DNA. Iron oxide nanoparticles have been used for many years to purify DNA samples. The DNA reversibly binds to the nanoparticle surface and can be readily removed from a solution by magnetic precipitation. Several workers

have modified this method to capture the DNA target more effectively using short oligonucleotides covalently attached to the magnetic nanoparticles.[10,11] Li *et. al.* used this method to capture amplicons from a PCR reaction via an oligonucleotide tag on the surface of magnetic nanoparticles. The amplicons were made with a biotin end-group and this was used to bind an alkalinephosphates enzyme for quantification.[12] Stelau *et. al.* combined this magnetic capture with a SERS readout, however, they used a SERS active surface not a SERS active nanoparticle.[13] More recently Hibbitts *et. al.* used a similar approach of capturing biotin and dye labelled amplicons with magnetic streptavidine beads then after purification reading the SERS signal associated with the dye labels using Ag nanoparticles.[14] Previous work has also been published showing the use of PCR and DNA to assemble nanoparticles.[15-17] In 2005, Deng *et. al.* showed that Au nanoparticles could be attached to large DNA chains though small oligonucleotides covalently bound to the AuNPs. The longer DNA chains were up to 4 micrometers in length.[18] Alivisatos *et. al.* formed AuNP dimers and trimers linked though DNA hybridization of short oligonucleotides on the NP surface.[19] Other workers have showed that PCR extension could be achieved on primers attached to AuNPs.[20,21] Zhao *et. al.* elaborated on this approach further by using different sized AuNPs with different primers (reverse primers and forward primers on small and large AuNPs respectively). The resulting nanostructure was composed of alternating large and small AuNPs. He also showed that the PCR reaction could be stopped at different cycle numbers to give different sized nanostructures.[22] Zhang *et. al.* showed that DNA could be used to assemble different types of NPs, however, this work focused on developing a tool for building complex nanostructures and not as a biosensor for target DNA or pathogen detection.[23] In addition, they did not combine SERS active nanoparticles with magnetic

nanoparticles as we have shown in our work. However, they did show that it was possible to perform PCR reactions on the surface of nanoparticles.

SERS has been used as an analytical tool for many years.[24] SERS active particles have been successfully employed as labels or probes in chemical assays,[25] immunoassays,[26-28] bacteria detection,[29] and DNA detection,[30] with the SERS peak intensity being correlated to the concentration of target species.[31] In the work presented here we have developed real-time PCR probes based on magnetic trapping and SERS detection. Short chain DNA tags were bound to magnetic nanoparticles (MNPs) and SERS active nanoparticles (SERS-NPs). These were then used to identify a target bacterial DNA sequence. The tags function as primers in a standard PCR reaction with the reverse primers and forward primers on the SERS-NPs and MNPs respectively. On binding to the target DNA the primers can be extended through a PCR reaction. Once extended the MNPs and the SERS-NPs are able to hybridize together through the extended DNA chain resulting in the formation of a composite nanostructure. This structure can be magnetically trapped and the SERS signal recorded using a desktop system. The process of extension and annealing can be repeated as in standard PCR reactions building up larger nanostructures. See figure 1 for an illustration of this process. The intensity of the SERS signal was related back to

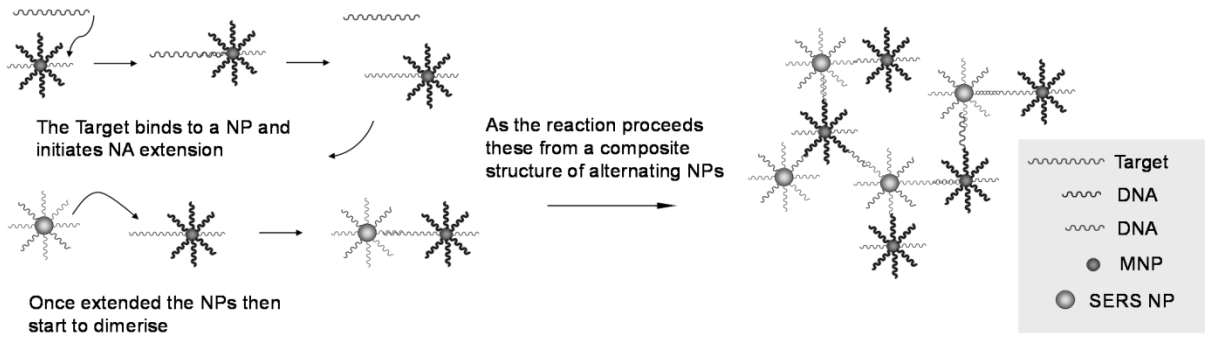

Figure 1: A schematic representation of the PCR mechanism using NP bound primers showing the

formation of the final nanocomposite structure

the concentration of the target DNA and gave a detection limit of 3 zeptomole (41 pg/L). Based on the signal to noise ratio and increasing the integration time it should be possible to detect less than 1 zeptomole (14 pg/L). The system offers a new approach for 'Real-Time PCR' and the potential to multiplex the readout, detecting several different DNA sequences in a single reaction.

2. Method

The desktop Raman spectrometer was an EZRaman-L system purchased from Enwave Optronics operating with 670 nm laser, 0.30 NA focusing lens and a 17 mW output measured with a LaserCheck power meter supplied by Coherent Inc.. UV–Vis spectra were recorded on a Jasco V-550 spectrometer. TEM data were recorded on a JEOL-2010 TEM operating at 200 keV with carbon film coated copper grids and scanning electron microscopy (SEM) data on a FEI Quanta 400 FEG. For details concerning the DNA sequences used throughout see the Online Resource file.

2.1 AuNP synthesis

AuNPs were synthesized following the citrate reduction method. This is one of the most common methods used for the synthesis of hydrophilic AuNPs.[32] Small AuNPs were made first and used as seed particles to grow the final larger AuNPs. For the small seed particles, trisodium citrate (50 mg) was dissolved in distilled water (5 mL) to produce a 1% solution. This was added to a refluxing solution of hydrogen tetrachloroaurate (20 mg) in distilled water (50 mL). The resulting solution went through a colour change from light yellow to deep red/purple. The heat

was removed after refluxing for 30 min. UV-Vis spectroscopy and TEM image analysis was used to characterise the solution.

Large AuNPs were synthesized by seed particle growth with citrate reduction. Hydrogen tetrachloroaurate solution (1 mL, 11 mmoldm^{-3}) was added to distilled water (32 mL) and brought to reflux with a condenser fitted. The seed solution from the above small AuNP synthesis was added to this (1 mL) followed closely by trisodium citrate (0.34 mL of 1% solution). The solution started to change to a blue colour after 30 s and a red colour after 1 min. After 10 min the heat was removed and the solution allowed to cool with stirring. The solution was characterised by UV-Vis spectroscopy, TEM image analysis and dynamic light scattering. The large AuNPs were mixed with 50 mg of 4-mercaptobenzoic acid, (MBA), dissolved in 5 mL of ammonia (1 moldm^{-3}).

The SERS spectra of the MBA-AuNPs was recorded using the desk-top system. The final solution (0.2 mL) was diluted with water (0.8 mL) and placed in the liquid sample holder. The holder held the sample so that the laser focus point fell in the centre of the solution. The laser exits the 105 μm diameter waveguide and passes through a collimation lens giving a beam width of 0.25 cm. The beam then passes through a lens with a focus length of 0.7 cm giving a spot diameter of 100 μm at is focus point. With this set-up, the laser effectively illuminates and collects a signal from a volume of 6.9 nL within the sample.

2.2 DNA coated MBA-AuNPs

DNA sequence C6-amino-1 (see Online Resource file) was used to coat the MBA-AuNPs by standard 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) coupling between the MBA-COOH group and -NH_2 group on the end of the DNA sequence. The MBA-AuNPs (118 μL)

were mixed with 1 μL of freshly prepared EDC (7 mg mL^{-1}). The DNA solution (2 μL of 100 pmol μL^{-1}) was added to this and the solution was left overnight with stirring then centrifuged and redispersed in 120 μL water. Care was taken when decanting the clear liquid from the AuNPs as these are readily redispersed. The product from this reaction is referred to as SERS-NPs.

2.3 IONP synthesis

The iron-oxide nanoparticles (IONP) were synthesized under an argon atmosphere. The standard procedure is outlined below. $FeCl_2$ (34.5 mmoles), $FeCl_3$ (69 mmoles) and deionised water (150 mL) were combined in a reaction flask. NaOH (5 $moldm^{-3}$) was added to adjust the pH of the mixture. The solution was subjected to continuous stirring during the reaction until the mixture became basic. The black precipitate formed was washed with deionised water and the pH adjusted to below 5 with glacial acetic acid. Finally, H_2O_2 (10 vol%) was gradually added until no further reaction occurred and washed with deionised water. This was judged as the point at which no further effervescence occurred on addition of fresh H_2O_2. The product was dispersed in fresh deionised water and dextran (MW=10,000 Da) was then added. After ultrasonic mixing, NH_4OH was added to bring the pH to 10. The mixture was then continuously stirred while being heated to 75°C and held at this temperature for 60 min. To remove excess dextran, the suspension was dialyzed using a membrane with a molecular weight cut-off (MWCO) of 10,000 Da. The suspension was then centrifuged at 6,000 rpm for 30 min to remove any large aggregates. Finally, the suspension was filtered through a 0.2 μm filter.

The IONPs were then coated with SiO_2. Briefly, the IONPs (5 mL, 3.6 mg mL^{-1}) were mixed in a 5 mL solution of water and 1g of polyvinylpyrrolidone (PVP10). The PVP used had

an average molecular weight of 10,000 Da. After 30 min, the IONPs were precipitated in ethanol and redispersed in 40 mL of distilled water. (3-Aminopropyl) triethoxysilane (APTES) (500 μL) was then added followed by six drops of concentrated NH_3. After 30 min, the product was magnetically precipitated and washed with five consecutive acetone and water washes. The product was finally dispersed in water (2 mL). The particles were characterised by TEM image analysis and energy-dispersive x-ray spectroscopy (EDX).

2.4 DNA coated IONPs

DNA sequence C6-carboxy-1 (see Online Resource file) was used to coat the IONPs by standard EDC coupling between the DNA -COOH group and -NH_2 groups on the IONPs. The IONPs (118 μL of 1 mg mL^{-1}) were mixed with 1 μL of freshly prepared EDC (7 mg mL^{-1}). The DNA solution (2 μL of 100 pmol μL^{-1}) was added to this and the solution was left overnight with stirring then magnetically precipitated and redispersed in 120 μL water. The sample was washed 5x with water. The product from this reaction is referred to as MNPs

2.5 PCR reaction

The real-time PCR analysis was performed on an Applied Biosystems 7500 Real-Time PCR system using DreamTaq™ Green PCR Master Mix K1081. This is a ready-to-use solution containing DreamTaq™ DNA polymerase, optimized DreamTaq Green buffer, $MgCl_2$ and dNTPs. All synthetic DNA was purchased from ThermoFisher Scientific. The target NA was synthetic, single stranded, 100 base pair DNA with a sequence taken from a bacterial plasmid (see Online Resource for more information). The primers were 44 base pairs long and consisted of a 20 base pair thymine spacer at the 5' end and a 24 base pair active primer section. Each

analysis was run for 40 cycles using the following protocol; Pre-Heat 95 ˚C for 10 min, Denature 95 ˚C for 15 sec, Anneal and Elongation 60 ˚C for 60 seconds. The primer concentration was 6 mg/L (10 picomoles) for each primer and the amount of target DNA varied from 0.14 mg/L (10 femtomoles) to 0.14 ng/L (10 zeptomoles). The total reaction volume for the PCR was 24 ul. Control assays were performed leaving out one component from the mix, either the forward primer the reverse primer or the target. The selectivity was investigated by the use of a none complimentary target DNA sequence, ABI commercial human genomic DNA (1 μg/L).

3. Results and Discussion

The AuNP synthesis proceeded as expected. The solution went through a colour change from light yellow to deep red/purple during the seed particle production. The UV-Vis spectrum for the solution was recorded from 300 nm to 900 nm and showed the characteristic SPR peak at wavelength 517 nm. TEM image analysis gave a NP diameter of 12 nm ± 1 nm. For the large AuNPs the solution changed to a blue colour after 30 s and a purple/red colour after 1 min. The UV-Vis spectrum for the solution was recorded from 300 nm to 900 nm and showed the characteristic SPR peak at wavelength 535 nm. TEM image analysis and dynamic light scattering analysis gave a NP diameter of 51 nm ± 2 nm. See Online Resource fig S1. The final SERS-NPs showed the characteristic spectra of MBA, this is dominated by the double peaks at about 1073 cm^{-1} and 1584 cm^{-1}. These can be assigned to the ν_{8a} and ν_{12} aromatic ring vibrations respectively.[33] See Online Resource fig S2.

The IONP synthesis produced a dark brown / black suspension that was stable for several days with no obvious precipitation. The suspension could be magnetically precipitated in a matter of seconds and readily redispersed using vigorous shaking or sonication. The synthesis

has several steps that have been optimised over time by our group. The first step is the pH precipitation of Fe_3O_4, the phase of the ironoxide was initially determined by X-ray diffraction[34,35] and later by simply observing the colour of the precipitate. A magnetic, dark black solid, is indicative of Fe_3O_4. At this stage the IONPs form clusters in solution which readily precipitate, dextran is added in order to stabilise the solution and to form well dispersed NPs. The dextran polymer coats the NPs and prevents aggregation and precipitation. These particles are stored for several months and used when required. In order to attach the DNA to the IONPs it is first necessary to add $–NH_2$ functional groups to the NP surface. This is achieved by using APTES, an amino functionalised silane that can self condense on the surface of the IONPs to produce a continuous SiO_2 layer with the desired $–NH_2$ functional groups. It was found that adding PVP to the NP surface prior to the APTES reaction allowed for the formation of a more even SiO_2 coating. The DNA primers were coated onto the IONPs by standard EDC coupling between the DNA -COOH group and $-NH_2$ groups. From TEM analysis the MNPs have a Fe_3O_4 core of 20 nm ± 3 nm and a SiO_2 shell of thickness 3 nm ± 1 nm. See Online Resource fig S3.

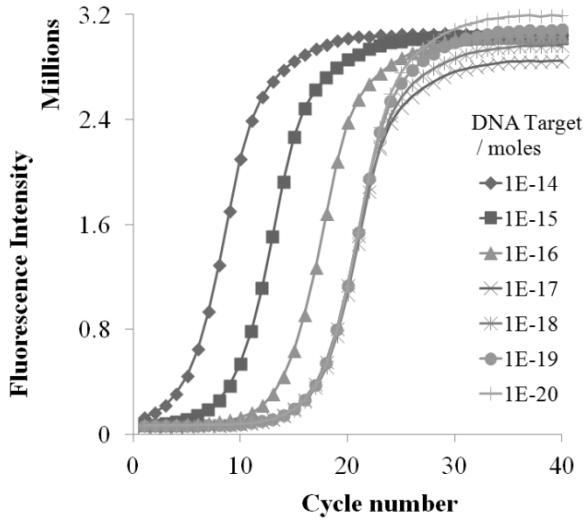

Figure 2: Results from the standard PCR assay for the NA target using free primers and SYBR Green

detection

Real-time PCR was conducted using standard protocols. Two assays were performed each using a range of target DNA concentrations. The first assay was the standard assay and acted as the control. This used the commercial real-time PCR kits with SYBR Green dye, the free primers and the target DNA sequence. The output from this assay was the SYBR Green fluorescence which increased as the assay proceeded and the target DNA was amplified, see figure 2. The second assay performed was the modified assay using the primers attached to the SERS-NPs and MNP. In this assay all the experimental detail was kept the same, however, the free primers were not added to the system, instead the primers attached to the NPs were used. For this assay there was no fluorescence as the SYBR Green dye was quenched by the NPs. At the end of the assay (40 cycles) the magnetic product was collected, washed, and the SERS signal recorded.

The control assay produced the expected amplification plots showing the exponential increase in fluorescence from the SYBR Green dye as the amount of amplicons increased, see figure 2. This showed that the primers were active towards the target sequence and allowed an estimation of the limit of quantification for the system. On substitution of the free primers with the primer bound NPs, (SERS-NPs and MNP), no SYBR Green fluorescence could be observed. This is attributed to the NPs quenching the dye signal. The amplicons in this assay are attached to the surface of the NPs and any double stranded DNA required for the SYBR Green fluorescence is bound between two NPs. This prevents the SYBR Green fluorescence signal and is further evidence for the formation of the NP composite structure. However, on magnetic trapping of the product from the reactions with different initial concentrations of target DNA, different SERS signal intensities were recorded. This is as expected for the system and indicates

that the assay was functioning as planned. Figure 3 shows the SERS signal intensity as a function of the initial target DNA concentration.

The amplification plot showing the results from the standard real-time PCR analysis can be seen in figure 2. It shows that a decrease in the target NA concentration from $1x10^{-14}$ moles (0.14 mg/L) in the reaction (total volume 24 ul) to $1x10^{-17}$ moles (138 ng/L) in the reaction, led to a quantifiable increase in the number of cycles required for the SYBR Green fluorescence to reach the threshold level. Any further decease in the target DNA level had no effect on the reaction. The time taken for the fluorescent signal to reach the threshold level for the $1x10^{-17}$ moles (138 ng/L) down to $1x10^{-20}$ moles (138 pg/L) was the same. For this reason the quantifiable detection limit for the NA assay performed using the DreamTaq™ Green PCR Master Mix was 10 attomoles (138 ng/L). The full plot showing the cycle threshold value (Ct) for different starting concentrations of the target DNA can be seen in the Online Resource fig S4. A control assay using no DNA target was also ran and showed no fluorescence. This can be seen in the Online Resource Fig S5.

For the SERS based detection the PCR primers were replaced with the NP bound primers. The MNP were coated with the primer for the target NA and the SERS-NP were coated with the primer for the NA sequence complementary to the target. The rest of the PCR protocol was left unchanged including the master mix and the cycling program. After 40 cycles the samples were remove from the PCR device. The magnetic material was collected and washed several times with distilled water and finally acetone. During these washing steps the SERS signal was recorded and showed a transition from the Raman signal dominated by SYBR Green in the initial PCR mix to a Raman signal dominated by the SERS-NPs. After washing the SERS signal intensity at 1077 cm^{-1} was correlated with the initial target NA concentration. The results can be

seen in figure 3. The data showed a good correlation in the full range of target NA concentrations used. The estimated detection limit with a 100 second integration time is around 3 zeptomole of target NA (41 pg/L), this is based on a signal intensity of around 900 and a noise level of 300. The full SERS spectra recorded for the magnetic PCR products after 40 cycles from samples with different initial concentrations of the target DNA can be seen in Online Resource fig S6. From these results it appears that the SERS-NP system is functioning as planned. The NA target anneals to the primers on the MNP and the primer undergoes extension during the elongation

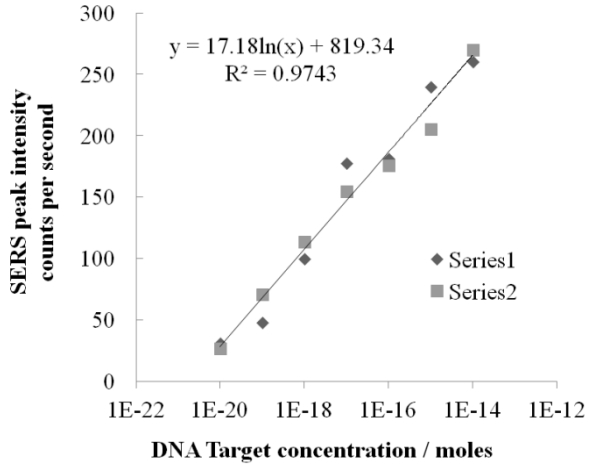

Figure 3: SERS signal response for different NA target concentrations in the PCR nanoparticle assay. Series 1 and Series 2 were recorded at different times with different signal integrations of 100 seconds and 10 seconds respectively, these are compared by dividing the signal intensity by the integration time

phase of the PCR cycle. Once completed the NA target is released during the denature step and annealed to a different primer (on the same MNP or on a different MNP) and the process repeated. Once a primer on the MNP has undergone extension the primers on the SERS-NP can hybridize with it forming dsDNA. After 40 cycles the MNP primers that have undergone extension can crosslink with the SERS-NPs forming a complex NP system that is both magnetically responsive and SERS active. The number of crosslinks in the system will be

determined by the initial concentration of the target NA and they in-turn will determine the number of SERS-NPs in the complex and the relative peak intensity in the SERS spectra. In the test assay performed a SERS signal was obtained from the 10 zeptomoles (0.14 ng/L) target NA sample. The control assay with no target DNA was also ran. For comparison the signal from the 10 zeptomoles (0.14 ng/L) sample and the blank control can be seen in figure 4. From the signal to noise ratio in this sample a detection limit of 3 zeptomole of NA can be estimated. For comparison the standard PCR reaction based on fluorescence for the same system had a detection limit of 10 attomoles, 3000 times less sensitive.

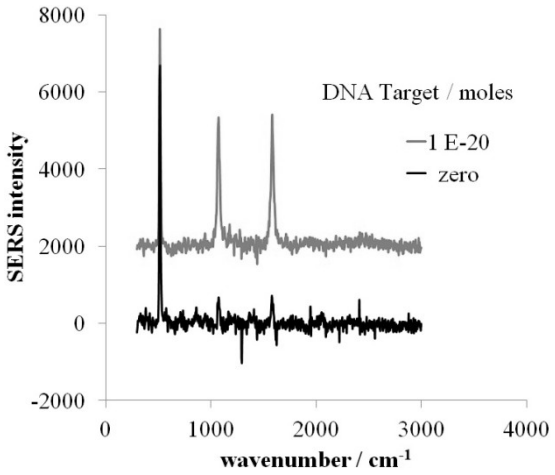

Figure 4: full SERS spectra for 10 zeptomoles of target NA after 40 PCR cycles using the nanoparticle protocol. Signal height at 1077 cm^{-1} is 3000 counts and the noise level is ± 100 counts. The peak at 516 cm^{-1} is from the Si surface and can be used as a reference peak

Future work will focus on recording the SERS signal as a function of both the PCR cycle number and the initial concentration of the target DNA. This should help to investigate the relative PCR efficiencies of DNA extension from a single NP and when two NPs are in close proximity, for example in SERS-NP and MNP dimer. Work will also begin on multiplexing the system and introducing SERS-NPs with different spectra with different DNA primers attached. It

is hoped that this will allow for the detection of multiple target DNA sequences in a single reaction.

References

[1] S.A. Bustin, Expert Rev. Mol. Diagn., 10 (2010) 713-715

[2] M. Maurin, Expert Rev. Mol. Diagn., 12 (2012) 731-754

[3] M. Li, Y.C. Lin, C.C. Wu, H.S. Liu, Nucleic Acids Res., 33 (2005) e184

[4] T. Higashi, Y. Nagaoka, H. Minegishi, A. Echigo, R. Usami, T. Maekawa, T. Hanajiri, Chemical Physics Letters, 506 (2011) 239-242

[5] H. Li, J. Huang, J. Lv, H. An, X. Zhang, Z. Zhang, C. Fan, J. Hu, Angew. Chem. Int. Edn., 44 (2005) 2-5

[6] J. Huang, X. Zhang, C. Wang, L. Wang, H. Li, X. Cao, A. Zhang, X. Li, C. Fan, J. Hu, Surface Review and Letters, 15 (2008) 757-762

[7] W. Yang, L. Mi, X. Cao, X. Zhang, C. Fan, J. Hu, Nanotechnology, 19 (2008) 255101

[8] B.V. Vu, D. Litvinov, R.C. Willson, Anal.Chem., 80 (2008) 5462-5467

[9] W. Wan, J.T.W. Yeow, Nanotechnology, 20 (2009) 325702

[10] N. He, S. Li, H. Liu, Methods Mol. Biol., 578 (2009) 393-402

[11] Y. Zhang, Y. Guo, P. Quirke, D. Zhou, Nanoscale, 5 (2013) 5027-5035

[12] Z. Li, L. He, N. He, Y. Deng, Z. Shi, H. Wang, S. Li, H. Liu, Z. Wang, D. Wang, J. Nanosci. Nanotech., 11 (2011) 1074-1078

[13] K.K. Strelau, A. Brinker, C. Schnee, K. Weber, R. Moller, J. Popp, J. Raman Spec., 42 (2011) 243-250

[14] S. Hibbitts, P.L. White, J. Green, G. McNay, D. Graham, R. Stevenson, Anal. Methods, 6

(2014) 1288-1290

[15] C.J. Loweth, W.B. Caldwell, X. Peng, A.P. Alivisatos, P.G. Schultz, Angew. Chem. Int. Ed., 38 (1999) 1808-1812

[16] I. Tokareva, E. Hutter, J. Am. Chem. Soc., 126 (2004) 15784-15789

[17] J. Elbaz, A. Cecconello, Z. Fan, A. O. Govorov, I. Willner, Nat. Commun., 4 (2013) 2000.

[18] Z. Deng, Y. Tian, S.H. Lee, A.E. Ribbe, C. Mao, Angew. Chem. Int. Ed., 44 (2005) 3582-3585

[19] A.P. Alivisatos, K.P. Johnsson, X. Peng, T.E. Wilson, C.J. Loweth, M.P. Bruchez, P.G. Schultz, Nature, 382 (1996) 609-611

[20] S.R. Nicewarner Pena, S. Raina, G.P. Goodrich, N.V Fedoroff, C.D. Keating, J. Am. Chem. Soc., 124 (2002) 7314-7323

[21] W. Chen, A. Bian, A. Agarwal, L. Liu, H. Shen, L. Wang, C. Xu, N.A. Kotov, Nano letters, 9 (2009) 2153-2159

[22] Y. Zhao, L. Xu, H. Kuang, L. Wang, C. Xu, J. Mater. Chem., 22 (2012) 5574-5500

[23] Y. Zhang, F. Lu, K.G. Yager, D. van der Lelie, O. Gang, Nature Nanotech., 8 (2013) 865-872

[24] S. Xu, X. Ji, W. Xu, B. Zhao, X. Dou, Y. Bai, Y. Ozaki, J. Biomed. Opt., 10 (2005) 031112, 1-12

[25] P.D. Enlow, M. Buncick, R.J. Warmack, T. Vo-Dinh, Anal. Chem., 58 (1986) 1119-1123

[26] J.D. Driskell, K.M. Kwarta, R.J. Lipert, M.D. Porter, Anal. Chem., 77 (2005) 6147-6154

[27] S. Xu, X. Ji, W. Xu, X. Li, L. Wang, Y. Bai, B. Zhao, Y. Ozaki , Analyst, 129 (2004) 63-68

[28] D.S. Grubisha, R.J. Lipert, H.Y. Park, J. Driskell, M.D. Porter, Anal. Chem., 75 (2003) 5936-5943

[29] P. Drake, P.S. Jiang, H.W. Chang, S.C. Su, J. Tanha, L.L. Tay, P. Chen, Y.J. Lin, Anal. Methods, 5 (2013) 4152-4158

[30] K. Faulds, W.E. Smith, D. Graham, Analyst, 130 (2005) 1125-1131

[31] X. Su, J. Zhang, L. Sun, T.W. Koo, S. Chan, N. Sundararajan, M. Yamakawa, A.A. Berlin, Nano Letters, 5 (2005) 49-54

[32] P. Drake, H.W. Chang, Y.J. Lin, J. Raman Spectrosc., 41 (2010) 1248-1253

[33] A. Michota, J. Bukowska, J. Raman Spectrosc., 34 (2003) 21-25

[34] A.J. Barker, B. Cage, S. Russek, C.R. Stoldt, J. Appl. Phys., 98 (2005) 063528

[35] P. Drake, H.J Cho, P.S. Shih, C.H. Kao, K.F. Lee, C.H. Kuo, X.Z. Lin, Y.J. Lin, 17 (2007) 4914-4918

Nanoparticle labels for pathogen detection through nucleic acid amplification tests

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DNA sequences

100 base-pair target DNA sequence taken from the E.coli-K.pastoris shuttle vector pPpHIS4

CTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTT
TGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGA

Forward primer (C6-carboxy-1)

COOH-$(Carbon)_6$-$(T)_{20}$-TCCCAACGATCAAGGCGAGTTACA

Reverse primer (C6-amino-1)

NH_2-$(Carbon)_6$-$(T)_{20}$-CTGCGGCCAACTTACTTCTGACAA

Supplementary Figures

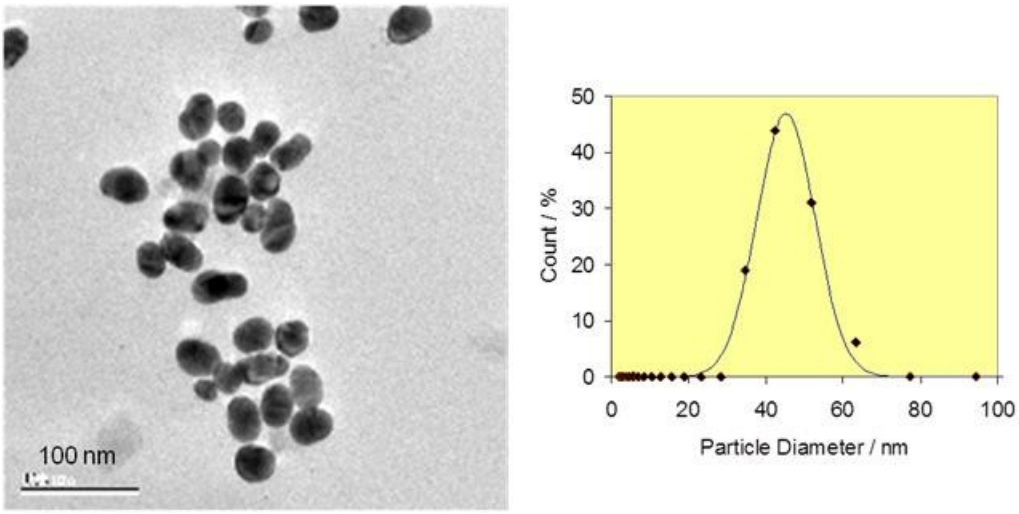


Fig S1: Showing the TEM image and DLS data for the large AuNPs

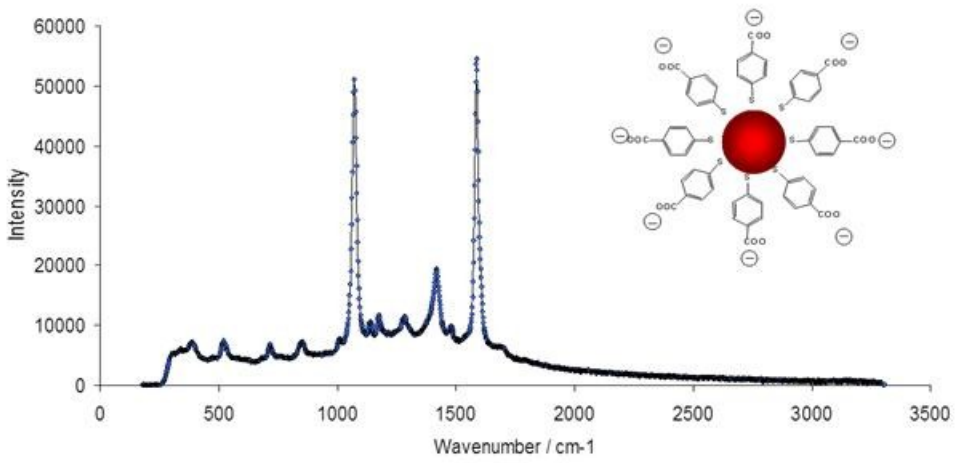
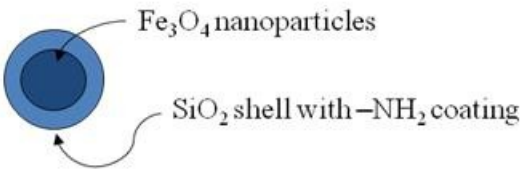
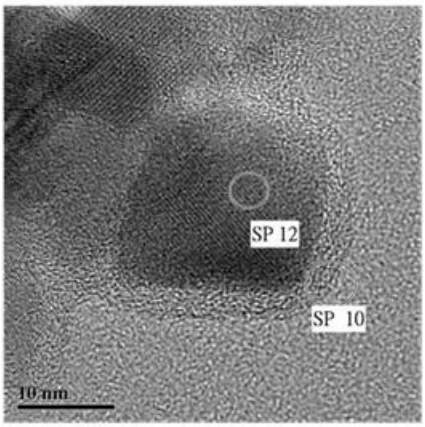


Fig S2: Showing the SERS spectra of the MBA-AuNPs. Inset shows an illustration of the NPs with the MBA coating



TEM image analysis showed magnetic core diameters of 20.3± 2.6 nm



SP12		
Element	Weight %	Atomic %
C K	47.97	69.66
O K	12.56	13.70
Si K	15.94	9.90
Fe K	7.63	2.38
Cu K	15.90	4.36

SP10		
Element	Weight %	Atomic %
C K	52.89	73.17
O K	11.75	12.21
Si K	16.29	9.64
Cu K	19.07	4.99

Fig S3: Showing the characterization of the IONPs. SP12 and SP10 correspond to different regions of the NP; SP12 includes the core and the shell while SP10 only includes the elemental composition of the shell

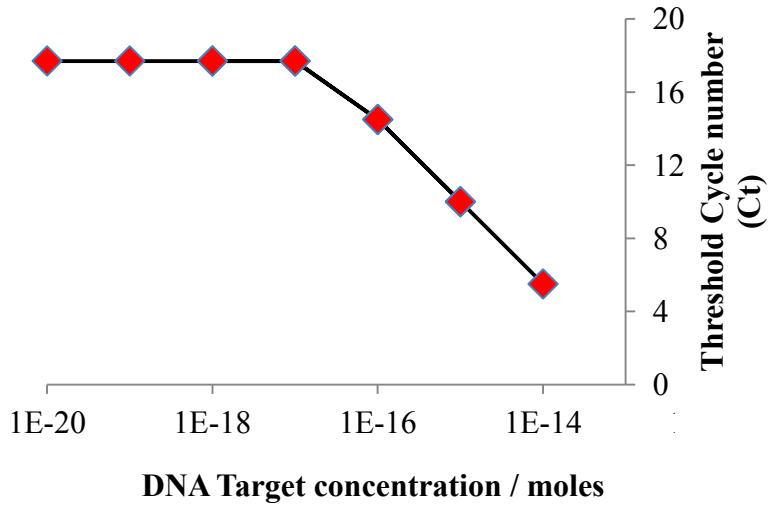


Fig S4: Results from the real-time PCR assay following the standard protocol using free primers. Curve showing the cycle threshold value (Ct) for different starting concentrations of the target DNA. The quantification region corresponds to the linear section of the curve from $1x10^{-14}$ moles to $1x10^{-17}$ moles of target DNA

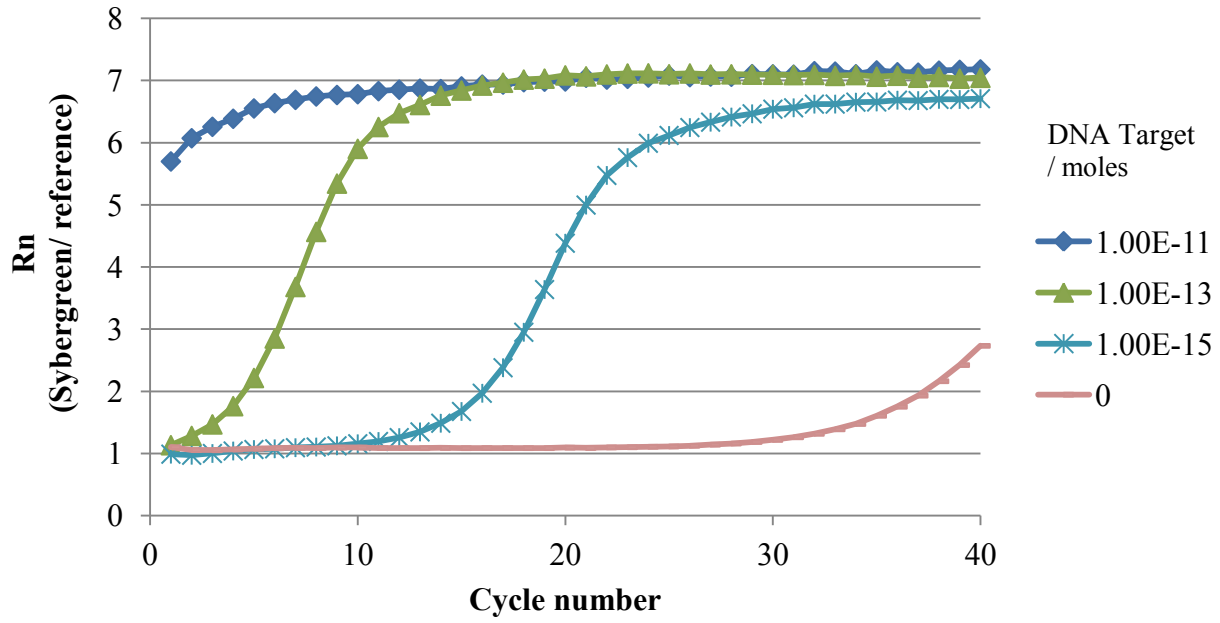


Fig S5: Results from the real-time PCR assay following the standard protocol using free primers. Duplicating the previous work but looking at high concentration DNA target and a blank control with no DNA target

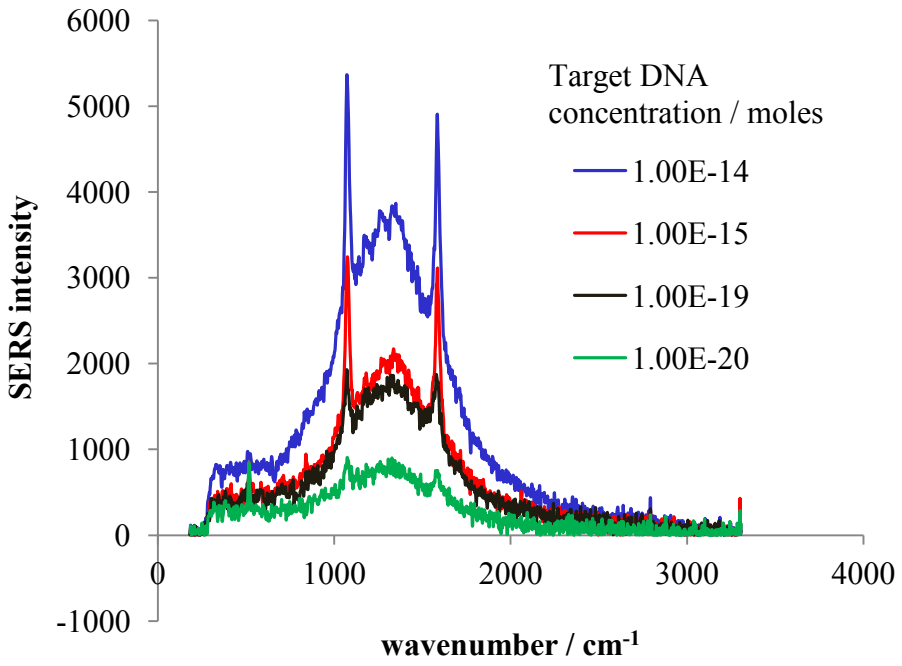


Fig S6: Showing selected results of the real-time PCR assay using the NP bound primers only (SERS-NP and MNP). The plot shows the full SERS spectra recorded with a 10 second integration time for the magnetic PCR products after 40 cycles with different initial concentrations of the target DNA